Studies on the Development of Pigment in the Eye and Testes of Wild type and Certain Mutants of *Drosophila pseudo-obscura*.

by

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Presented to the University of Edinburgh as a Thesis for the Degree of Ph.D. May 1937.

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Studies on the Development of Pigment in the Eyes and Testes of Wild type and Certain Mutants of *Drosophila pseudo-obscura*.

Introduction.

There is an increasing abundance of evidence in genetic literature that the genes are capable of precise regulation of developmental processes. One of the fundamental problems of genetics is to find out how this regulation is brought about. This is an embryological problem envolving the collection of data which show the normal sequence of events in development, and in mutant types the times when deviations from the normal course take place and the nature of these deviations.

Goldschmidt was one of the first to attempt to correlate the facts of genetics and the facts of embryology. His important contributions based on observations on Lymantria are summarized in "Physiologische Theorie der Vererbung" (1927). Goldschmidt enlarged the conception of the gene and its action into a general theory of heredity. The fundamental basis of this theory is the conception of differential and balanced reaction velocities of the genes proportional to the specific valencies of the genes Morgan (1926) in his critical paper on gene action supported the view of several geneticists that genes may be considered enzymes. He discussed the possibility that the process by which genes themselves increase is the same as that by which they affect

development and that the genes are composed of the same substances which they set free. Jollos (1934) while trying to explain the parallelism of modifications and mutations produced by heat treatment put forward the same suggestion.

Plunkett (1926) working on Drosophila discussed genes which bring about certain effects by causing the more rapid production of catalysts which oppose normal reactions during the ontogeny of the organism. A similar suggestion arose from the analysis of gene action in maize made by Brink (1929).

Goodrich (1927) found that in Oryzias certain genes produce their different colour effects by altering pigment deposition quantitatively.

Ford and Huxley (1928) investigating rates of development of eye colour in Gammarus elaborated the idea that certain genes bring about their effects by altering rates of development and also by affecting the time of onset of gene action.

A study of gene action during the various processes of ontogenic development should throw light upon the complex problem of gene structure. The object of the present work has been to make a detailed examination of the eye pigment of Drosophila pseudoobscura at various stages of development to determine when and how the genes which influence eye colour production bring about their effects. Eye colour was chosen because genes responsible for eye colour

mutations condition specific reactions late in development, when the fly approaches the imaginal stage. At this time structures are readily identified and the material easily handled.

This thesis is presented in the form of four independent papers, one published, one in press and two ready for press.

The first paper deals with the sequence of colour changes observed in the eyes of pupae of Drosophila pseudo-obscura of known ages.

The second paper based on histological findings describes the distribution of pigment granules in the eyes of wildtype and several mutants at various points in the developmental process.

The third paper describes three new eye colour mutants which appeared while the above work was in progress. The new genes are located and their genetic behaviour in combination with other eye-colour genes is discussed.

The fourth paper includes data on testis colour which accumulated while the eye colour data were being recorded and which appeared to be ins ome way connected with the observations on eye colour.

The technique used for each step of the work is described in the papers concerned. An attempt was made to study pigment distribution by photographic means. The method of investigation and results obtained are given in a short appendix.

Historical Summary.

Eyes of Diptera have been studied from many points of view. Weismann (1864) described their early development, Hickson (1885) and Lowne (1895) their adult structure. More recently Krafka(1924) and Chen (1929) have outlined the development of eyes in D. melanogaster. Richards and Furrow(1925) Johanson (1924) and Casteel (1929) have described their adult histological structure.

Morgan and Bridges (1913) Bridges and Morgan (1919) and (1923) and Bridges (1919) have discovered numerous eye colour mutations in D. melanogaster. They have located the genes responsible for these mutations and studied their inter actions. Lancefield (1922) has made similar discoveries in D. pseudo-obscura. Crew and Lamy (1932, 1934, 1935) have described other eye colour mutations in the latter species and have discussed their interactions.

Schultz investigated eye pigment in D. melanogaster chemically and spectroscopically (1929) (1935) and from the standpoint of development (1932) and (1935). Laki (1935) and Hertwick (1931) made some observations on the chemistry of eye pigment in D. melanogaster. Hertwick (1931) and Mainx (1935) also offered some suggestions about the activity of genes during the development of pigment in the eyes.

Beadle and Ephrussi (1935 a & b, 1936, 1937 a & b) have used transplantation experiments on

D. melanogaster to show that eye pigment is governed by diffusible substances present in all tissues of the wild type fly and that mutant genes act by removing specific parts of these substances.

Brown and Hall (1936) have made physiological studies concerning the response to light by various eye colour mutants of D. melanogaster.

Acknowledgment.

The author is greatly indebted to Prof. F.A.E. Crew, Ch.B., D.Sc., M.D., F.R.S.E., Director of the Institute of Animal Genetics, University of Edinburgh, for the scientific hospitality extended to her, and also for his interest, encouragement and advice during the progress of the work. Grateful acknowledgment is also due to other members of the staff and research students in the institute for help received from them, to Miss Rowena Lamy and Dr. P.C. Koller for valuable advice and criticism and to Mr H.D. Slack for help with the photography and other technical problems.

Observations on Eye-Colour Development in *Drosophila pseudo-obscura*.

Published in the Journal of Genetics,
vol. XXXII. No. 2 pp. 183-187.
April 1936.

OBSERVATIONS ON EYE-COLOUR DEVELOPMENT IN DROSOPHILA PSEUDO-OBSCURA.

Introduction.

In the course of three papers Crew and Lamy (1932- 1934, 1935) have described five mutant eye colours of Drosophila pseudo-obscura, namely three allelomorphs of purple, eosin and sepia, and five combinations of these with vermillion and one with orange. They have suggested an hypothesis (1935) for explaining the combined action of eye-colour genes with reference to the time of deposition of pigments during pupal life. They believe that each gene influences eye-colour development at a time characteristic for itself, and that each gene produces its effect independently of the others, and hence that the effect of the presence of several genes is cumulative. There is, properly speaking, no real interaction between genes, and no modification of effects. As the observations on which this hypothesis was based were admittedly superficial, these authors have suggested that a detailed study of developing pupal eyes should be made.

In a recent paper Jack Schultz (1935) tabulates the colour changes in the pupal development of the different eye-colour types of Drosophila. In most respects the present findings on Drosophila pseudo-obscura are in agreement with Schultz; in some they

differ. Although he does not mention a species, it is assumed that Schultz made his observations on Drosophila melanogaster, and that the differences pointed out in this paper are specific ones.

Material and Technique.

Flies for this study were obtained from the stocks used by Crew and Lamy. These were inbred for several generations. Stocks of orange-purple^2 and orange-purple^3 were prepared, and added to the list for study. Orange-purple^2 and orange-purple^3 are indistinguishable from vermilion-purple^2 and vermilion-purple^3 respectively.

Pupae for study were isolated not more than 1 hour after pupation and incubated at 25°C. for definite periods, after which dissections were made under a Zeiss binocular dissecting microscope. At first series of pupae each about 24 hours older than the other were dissected at the same time. Very early it became obvious that colour first appears in pupal eyes at about 96 hours after pupation, and that practically no change takes place between the 144th hour and emergence. For this reason all the observations recorded in this paper were made on pupae between 90 and 148 hours after pupation. As many mutant types as possible of similar ages were examined at one time in order to make accurate comparisons.

In order to know the time at which colour appears in the eyes and the times at which any changes take place, great care was taken in isolating pupae as soon after pupation as possible. In order to do this mature larvae were transferred from their culture vials to Petri dishes lined with moist filter paper on the centre of which had been placed a little fresh food. A ring of dissolved yeast was spread around the edge of the paper to attract the larvae away from the food as it was found that the larvae of D. pseudo-obscura have a strong tendency to pupate within the food mass rather than upon the paper. These Petri dish cultures were incubated at 25°C. and examined hourly when all the pupae on the filter paper were collected. Pupation was taken to mean the moment at which the anterior spiracles are everted and the larvae cease moving. The pupae were isolated in small gelatin capsules punctured with a fine needle to let in air. Each capsule contained a small amount of food wrapped in tissue paper to keep the pupa moist.

All the pupae were dissected and examined under a binocular dissecting microscope using the same source of illumination; an electric lamp with a copper sulphate solution filter.

As all development takes place in the dark in the incubator, control pupae were kept in the light in

the constant temperature room to determine whether any differences due to the presence of light could be detected. As the times of pigment deposition were the same as well as the times of subsequent changes in colour, it was decided that the absence of light in the incubator does not affect pigment development in D.pseudo-obscura. Some individual variation in the rates of development was observed between pupae isolated simultaneously and incubated under identical conditions. This variation has been taken into account and the hours given for each step represent the hours at which the majority of the pupae examined passed through the various stages.

Observations.

The data obtained from the dissection of pupae are included in the following table. Some mutant types (vermilion, eosin purple[3] and combinations involving these) are extremely inviable and the pupae are very difficult to obtain or to keep alive for the required periods. Hence the small number of records of these types.

RECORDS of EYE COLOUR in PUPAE of VARIOUS AGES.

90-108 hrs.	109-119 hrs.	120-132 hrs.	133 hrs.—	Post pupal colour.
yellow → tan 26	tan → red 23	Opaque orange (Dark bristles) 18	Orange → dark brownish red 8	Dark red.
yellow → tan 10	tan 20	tan (Dark bristles) 24	light brown 8	reddish brown.
yellow → tan 6	tan → red 15	similar to wild type (Dark bristles) 8	slightly lighter than wild type 10	similar to wild type
yellow → tan 3	tan 3	reddish tan (dark bristles) 6	golden brown 4	similar to pr^1
yellow → tan 10	tan → red (slight) 10	golden brown (dark bristles) 14	golden brown with suggestion of red 8	similar to pr^1 & pr^3 more red.
yellow → tan 17	tan → red 13	Opaque orange - more yellow than wildtype. 29	more yellow than wildtype 10	Brownish red → black
0	No colour → yellow 2	Colourless → Orange (bristles dark) 16	Orange 5	Orange
No colour	No colour → yellow	Colourless → orange (bristles dark)	Orange	Orange
3	17	20 Colourless → dilute orange	7 dilute orange	dilute orange
0	0	6 Colourless	3 Colourless-- dilute orange	~~very~~ dilute orange.
0	0	3 Colourless → dilute orange	dilute orange	Very dilute orange
0	0	15 Colourless	5 Colourless-- dilute orange	very dilute orange
0	0	5 Colourless	14 Similar to (or pr 3)	Similar to or pr^3 and v pr^3
0	0	5 Colourless → lime yellow	8 Lime yellow	Lime yellow → brown
		5	5	

(Numbers refer to number of pupae dissected).

Discussion.

In Drosophila pseudo-obscura there are two distinct phases in eye-colour development. In wildtype the early phase is similar to that described by Schultz, in that the first colour to appear is yellow which becomes tan almost immediately. The tan changes to red between 108 and 112 hours after pupation. Schultz also observed this change which, in the species studied by him, coincides with the darkening of the bristles and the beginning of the late phase of pigment formation. In Drosophila pseudo-obscura this darkening of bristles does not take place until 120 hours after pupation, and at the same time a second phase of pigment deposition begins. During this late phase the eyes become increasingly opaque and orange in colour, suggesting that both red and yellow pigments are laid down simultaneously. Toward the end of the pupal period (from about the 142nd hour) and during post-pupal life, the colour of the wildtype eye becomes darker and duller, suggesting a second chemical change in the pigment already present.

The observations made in the course of this study support the idea of Crew and Lamy (1935) that the sequence of pigment development in each mutant type of eye corresponds to the development of pigment in the wildtype eye, and that the action of

each mutant gene is to suppress some portion of the typical process without altering the time relations of events involved.

Purple^{2} is very similar to wildtype both in pupal development and in adult colour, the only difference being that after the 120th hour slightly less pigment appears to be added.

The development of sepia is identical with that of wildtype until the 120th hour. After this hour the eyes of sepia become opaque and orange in colour. This orange is more yellow than the orange which appears in wildtype during this phase, suggesting that sepia differs from wildtype in that after the 120th hour no more red but considerable yellow pigment is laid down.

Eosin, purple^{1} and purple^{3} are indistinguishable from each other and from wildtype, purple^{2} and sepia until the 108th hour. Purple^{1} remains tan and does not seem to acquire additional pigment during the pupal period. In purple^{3} the tan colour is partially changed to red at about the 120th hour, after which a very little pigment (mostly if not all yellow) is added. Eosin is very similar to purple^{3}. It takes on a slight flush of red before the 120th hour, and afterwards becomes a rich golden brown, indicating the addition of yellow but of little if any red pigment.

Orange and vermilion remain colourless until about the 120th hour, after which both yellow and red pigment appear to be deposited gradually. After the first transitory yellow stage the colour of both these mutants is always orange, increasing in intensity as the pupa becomes older. Thus it seems that the vermilion and orange genes act by suppressing the early phase of pigment development, but allowing the late phase to proceed up to the point where the wildtype eye darkens and becomes more brown. Schultz states that in scarlet-like eyes the first pigment appears just before the time when the wildtype eye changes from tan to red. In Drosophila pseudo-obscura no colour appears in either vermilion or orange eyes before the time of the onset of the late phase of pigment development in the wildtype eye.

The effect of the presence of combinations of two mutant eye-colour genes on development is shown graphically in Fig. 1. Each gene as a suppressor is independently effective during a specific period, and typical development of pigment as in wildtype takes place whenever neither inhibiting influence is being exerted. Whenever orange or vermilion genes are present no pigment develops before the 120th hour after pupation. When the gene for purple[1] is present no development of colour takes place after the 108th hour of pupal life. Since this accounts for the entire period of pigment deposition,

vermilion-purple^{1} and orange-purple^{1} are colourless. In vermilion-purple^{2} and orange-purple^{2} only that pigment normally laid down in the purple^{2} eye after the 120th hour is allowed to develop, a dilute orange eye resulting. Similarly, in orange-purple^{3} vermilion-purple^{3} vermilion-eosin and vermilion-sepia, no colour appears before the 120th hour, and thereafter only the amounts of yellow and red normally laid down by purple^{3}, eosin and sepia respectively during that period develop.

In all except vermilion and orange, and in most combinations including vermilion and orange, the eyes darken and become more or less brown toward the end of the pupal period and during postpupal life. Schultz has pointed out that the three colours observed in Drosophila eyes are closely related chemically, and it seems possible that this late development of brown is due to a chemical change in the pigment already present.

From the observations made during this study it seems possible that the amount of brown is somehow related to the proportion of yellow to red in the pupal eye. Sepia, in which the proportion of yellow appears to be greatest, becomes darkest, whereas wildtype and purple^{2} contain considerably more red which seems to mask the brown. Purple becomes

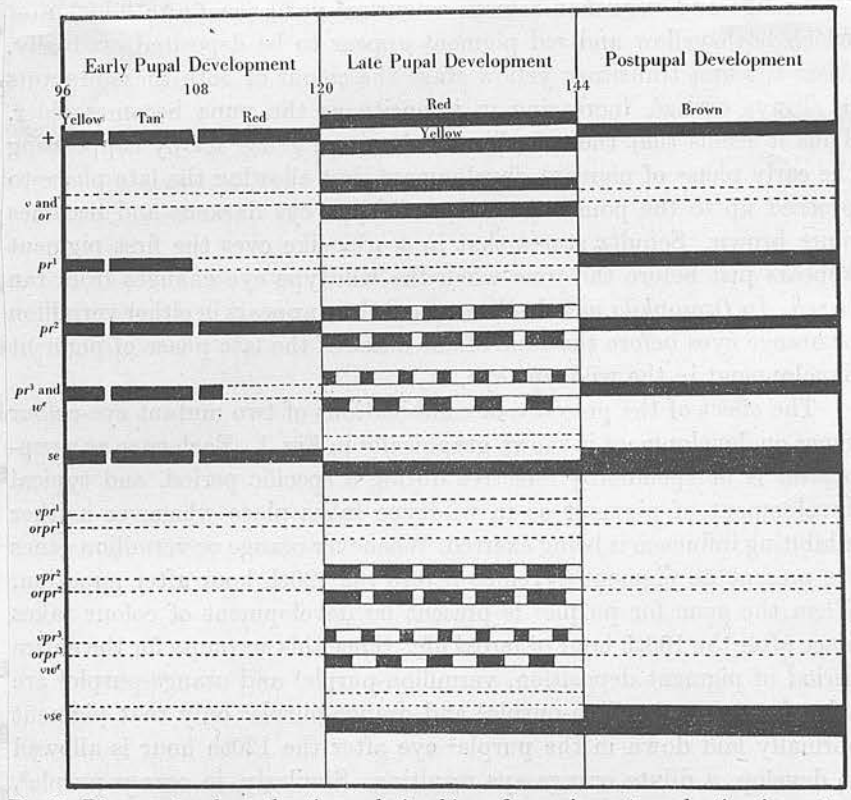

Fig. 1. Diagram to show the time relationships of eye-pigment production in mutant eye-colour types of *Drosophila pseudo-obscura*. Solid lines indicate formation of pigment. Dotted lines indicate suppression of pigment development. Broken lines indicate partial suppression of development, the amount of black indicating the relative quantity of pigment formed.
+ = wildtype; *v* = vermilion; *or* = orange; pr^1 = purple^1; pr^2 = purple^2; pr^3 = purple^3; w^e = eosin; *se* = sepia.

very dark in spite of the small amount of pigment present, all of which is yellow. Purple^3 and eosin increase in redness as well as becoming more brown after emergence. Vermilion, eosin and orange-purple^3, as well as vermilion-purple^3, which contain only a small amount of yellow pigment at the time of emergence, also become somewhat red during early postpupal life. It might be argued that the pigment laid down during the early phase of pigment formation has the property of turning brown, while that laid down after the 120th hour changes partially

to red. This cannot be accepted because vermilion-sepia, in which no colour appears before the 120th hour and which contains a large quantity of yellow but no red pigment at the time of emergence, develops nearly as intense a brown as sepia. Extreme darkening appears to be a property of yellow pigment in the presence of the sepia gene, but no satisfactory explanation for this peculiar influence of the sepia gene can be suggested until histological observations are made.

Having determined the sequence of events in the development of pigment it is intended to make a comparative histological study of each of these mutants at the times when the important changes occur. This, it is hoped, will give a measure of the amount of each pigment present in each mutant at each stage of development, and help to make clear which events are the result of the deposition of pigment granules and which are due to chemical changes in the granules already present.

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An Histological Analysis of Eye Pigment Development in *Drosophila pseudo-obscura*.

AN HISTOLOGICAL ANALYSIS OF EYE PIGMENT DEVELOPMENT IN DROSOPHILA PSEUDO-OBSCURA.

Introduction.

It is generally accepted that the function of the mutant genes which affect eye colour in Drosophila is to produce either quantitative or qualitative changes in the normal development of eye pigment. Wright (1932) believes that these genes act by interfering with some part of a chain of reactions which give rise to eye-colour characteristic of the wild type. Mainx (1935) suggested that recessive eye colour genes (with the exception of sepia) reduce the total amount of pigment, and that the degree of reduction is characteristic for each gene. Transplantation experiments of Beadle and Ephrussi (1935, 1936, 1937a and b) have led them to the view that certain eye colour mutants lack specific substances which are necessary for the development of additional pigment present in wild type eye colour. Johannson (1924) and Schultz(1935) studied the histology of the adult eye of D. melanogaster and classified the mutant eye colours according to the distribution of granules in their pigment cells. Schultz (1932) had previously noted the time of pigment deposition and subsequent changes in wild type and some mutants of D. melanogaster. In an earlier paper the author (1936) described in detail similar colour changes in the eyes of developing pupae of D. pseudo-

obscura. Previous work in this laboratory (1932, 1934, 1935) by Crew and Lamy make it appear that each mutant eye colour is the expression of a gene which acts to suppress normal development during a certain period of time, allowing the rest of the development to proceed as in wildtype.

The object of the present work has been to make a comparative histological study of pigment, in normal and mutant eyes of D. pseudo-obscura at various stages of development, in order to determine when and how mutant genes affect the normal process of colour formation.

The author wishes to thank Professor F.A.E. Crew and his colleagues at the Institute of Animal Genetics for facilities for work, helpful suggestions and encouragement during its progress.

TECHNIQUE AND METHODS.

Pupae of wildtype and eight eye colour mutant types orange (or), vermilion (v), sepia (se), three allelomorphs of purple (se), eosin (w^e) and white (w^5) were isolated. Having previously determined the times at which pigment appears in the eyes as well as the times at which colour changes take place, pupae of the appropriate ages were dissected and fixed. Carnoy Lebrun, Eltringhams fluid and alcoholic Bouin were used as fixatives. Carnoy Lebrun gave the best results though Bouin was also useful. Material was fixed for

not more than ten minutes then washed in absolute alcohol (to which a few drops of iodine had been added when Carnoy Lebrun was used as a fixative). The material was never allowed to remain in the alcohol for more than ten minutes. It was then cleared and embedded by the quick cellodin paraffin method of Peterfy (1928). Sections were cut 7-μ in thickness and mounted without staining in order to study the distribution of the pigment granules. These histological preparations were compared with fresh smears of the eyes, and it was found that the colour of the pigment granules was practically unaffected by fixation and clearing. A few sections were stained with Ehrlichs haemotoxylin and eosin for comparison with the unstained material. Micro-photographs of sections and smears were used to compare the relative sizes of the pigment granules.

DESCRIPTION.

The eye colour of Diptera as well as other insects as described by various investigators (Hickson, 1885, Lowne, 1895, Eltringham, 1919) is due to the presence of pigment granules in definite pigment cells which form sheaths around the omatidia. In Drosophila (Johannson, 1924) each sheath is composed of a pair of primary pigment cells surrounding the pseudocone and about twelve secondary pigment cells around the retinulae (Plate I, fig. a). Adjacent omatidia share the same secondary pigment cells. Johannson (1924)

described a third type called basal pigment cells which were supposed to lie at the bases of the omatidia. Hertwick (1931) denied the existence of these. During this study small clusters of yellow and brown granules were found lying at the bases of the omatidia in some pupae. These have no connection with the secondary pigment cells, nor could their connection with any other cells be determined. Aside from these clusters of granules there is no indication of a third type of pigment cells. These observations agree with Hertwick (1931. The secondary pigment cells are thicker at their proximal ends, and these thickenings contain more granules than do the other portions of the cells. Possibly these thickened ends of the secondary cells are what Johannson (1924) and Casteel (1929) described as basal pigment cells. In older pupae amd adult flies some granules are present below the basal membrane. As these are identical in appearance with the granules in the secondary pigment cells, and possibly lying within processes of these cells, they are included in the description of granules in the secondary pigment cells.

The pigment cells originate as the pyramidal supporting cells described by Krafka (1924). By 96 hours after pupation the eyes are completely formed, all the pigment cells are present, and in wild-type the first coloured granules appear. Aside from the deposition of pigment granules the only further

changes to take place are the cupping of the pseudocones late in pupal life and the lengthening of the omatidia. This lengthening causes the secondary pigment cells to stretch until they form thin sheaths around the retinulae. At about 168 hours after pupation the adult fly emerges from the pupa case.

At two distinct times during the pupal period large numbers of coloured granules are laid down in the pigment cells. These times mark the beginning of two phases of pigment development.

I. The early phase of pigment development

In the eyes of 104 hour old pupae about 8 hours after the onset of pigment formation, of wild type, sepia and three allelomorphs of purple, two kinds of granules (yellow and brown) are apparent in the primary and secondary pigment cells (Plate I, fig. b). These granules are of uniform size in both types of cells. They are undoubtedly yellow when they appear, and gradually become brown during this phase. Small clusters of yellow and brown granules are present at the bases of the omatidia, but their connection with any cells could not be determined. The eyes of eosin pupae of this age and also a little older contain the same yellow and brown granules in similar numbers as in wildtype in the primary pigment cells. In the secondary pigment cells there are fewer of the same type of granules. No yellow and

brown granules were present at the base of the omatidia (Plate I., fig c.) In vermilion, orange and white pupae of this age no pigment granules of any kind are present either in the primary or secondary pigment cells (Plate I, fig. a.) Stained sections of orange and vermilion as well as white pupae of similar ages show that the eyes are fully formed as in wildtype, and that both types of pigment cells are present in the usual numbers. Hertwick (1931) describes a white mutant in D. melanogaster which lacks pigment cells entirely. This is not the case in white^{5} (w^{5}) of D. pseudo-obscura.

In the eyes of wildtype, sepia and purple^{2} pupae between the ages of 110 and 120 hours, the number of granules is practically the same as that noted in younger pupae (Plate I., fig. e.) The granules in the primary cells are all yellow and brown. In the secondary cells there are numerous yellow and brown granules with red ones amongst them. In older pupae there are more red granules and correspondingly fewer yellow and brown ones. These observations indicate that during the early phase of development the yellow granules originally laid down in the secondary pigment cells undergo two colour changes by which they become brown, then red. Similar yellow granules in the primary cells become brown but never red.

In eosin pupae of this age, although there are

few granules in the secondary pigment cells, they become red as in wildtype. The granules in the primary pigment cells also behave normally.

In vermilion, orange and white[5] pupae under 120 hours old no coloured granules of any kind were present in primary or secondary pigment cells.

In pupae of purple[1] and purple[3] at the end of the early phase of development, the eyes contained only yellow and brown pigment granules in both types of pigment cells. No red granules were present (Plate I. fig. d.)

The histological data showing the differences in distribution of eye pigment in the various mutants during the early stages of development are summarised in Table I.

Table I.

Early Phase of Pigment Development (96-120 hours after pupation).

	96-108 hours after pupation.	109-120 hours after pupation.
wildtype	P. yellow and brown granules. S. " " "	P. yellow and brown granules S. red and brown granules present, more red than brown in older pupae
sepia (se)	P. same as wildtype S. " " "	P. same as wildtype S. " " "
purple^{1}(pr^{1})	P. same as wildtype S. " " "	P. same as wildtype S. brown and yellow granules only
purple^{2}(pr^{2})	P. same as wildtype S. " " "	P. same as wildtype S. " " "
purple^{3}(pr3)	P. same as wildtype S. " " "	P. same as wildtype S. same as purple^{1}
eosin (w^{e})	P. same as wildtype S. fewer yellow than in wildtype	P. same as wildtype S. few granules present, mostly red at the end of this period
white (w^{5})	both types of pigment cells present, no granules in either type	no granules present
vermilion (v) and orange (or)	same as white	same as white

P = primary pigment cells
S = secondary pigment cells.

II. The late phase of pigment development (over 120 hours after pupation.) In the eyes of vermilion (v) and orange (or) pupae over 120 hours after pupation the secondary pigment cells contain numerous orange granules (Plate I, fig. g.) In younger pupae these granules are yellowish, in older pupae they are

more red than yellow. This gradual increase in redness as well as in the size of the granules continues into adult life. No granules are present in the primary pigment cells, and no clusters of yellow and brown granules at the bases of the omatidia.

In the eyes of wildtype in the late phase of pigment development there is no change in the number or colour of the granules present in the primary pigment cells (Plate I, fig. h.) There is a marked increase in the number of granules in the secondary pigment cells. The additional granules are similar in number and colour to the granules present in the secondary pigment cells in the eyes of orange and vermilion pupae over 120 hours old. These new wildtype granules increase in size and redness as the pupae become older and after emergence in the same manner as the similar granules in orange (or) and vermilion (v) eyes.

The eyes of purple^{2} (pr^{2}) pupae during this late phase of development are similar to wildtype in that additional granules are present in the secondary pigment cells, and that the granules in the primary pigment cells remain unchanged. The difference from wildtype becomes obvious in older pupae (Plate II., fig. f.) The late phase granules in purple^{2} (pr^{2}) eyes are not as red as those of wildtype. It is difficult to decide if they are as large.

In the eyes of purple^{3} (pr^{3}) pupae just over 120 hours after pupation many of the early phase granules in the secondary pigment cells are still brown though some of them are red (Plate I, fig. i.) In older pupae the majority of the early phase granules in the secondary pigment cells have become red. In pupae of 140 hours additional yellowish granules are also present in the secondary pigment cells (Plate II, fig. g.) The granules in the primary pigment cells are yellow and brown as in the early phase of development.

The eyes of purple^{1} (pr^{1}) pupae over 120 hours old do not differ from the eyes of younger pupae. There are no additional granules in the secondary pigment cells, and the colour of the granules previously laid down in both types of pigment cells remains unchanged. Sections of purple^{1} (pr^{1}) pupae 140 hours old show some red granules scattered among the brown ones in the secondary pigment cells (Plate II, fig e.) Thus the transition to red which takes place in the early phase granules of the secondary cells of wild-type at about 115 hours in purple^{1} begins very late in pupal life. This colour change continues into adult life. In these adults the number of red granules increases and the number of brown ones decreases proportionally, indicating that these processes are causally connected. By two weeks after emergence all of the brown granules have become red, showing that tha transition is complete.

An accurate understanding of late pupal development of the purple alleles is difficult for the reason that the presence of early phase granules obscures the granules laid down later. Therefore it was decided to make a study of orange purple^{1}, orange purple^{2}, vermilion purple^{2} and orange purple^{3} where no early phase granules exist. The histological structure of the eyes of old and young adults carrying these colour combinations was compared with that of orange and vermilion adults of similar ages (Plate II, figs. i, j, k.) It was found that the granules in the eyes of orange purple^{2} (or pr^{2}) and vermilion purple^{2} (v pr^{2}) are much smaller and less red than those in orange and vermilion eyes of flies of the same age. The granules in orange purple^{3} are smaller and less red than those of orange purple^{2}. A corresponding increase in size of granules was noted in orange, orange purple^{2} and orange purple^{3} as the flies become older. The eye of an orange purple^{1} adult shows a complete absence of pigment granules, though the cells are present in the usual number.

The deposition of pigment granules in the sepia eye follows the same course as wildtype until after the 120th hour at which time there are numerous red granules in the secondary pigment cells. During the late phase of development additional granules of a yellowish colour may be observed in the secondary

pigment cells among the red ones (Plate II, fig. d.) These granules remain yellow and relatively small during pupal and adult life instead of becoming red as in wildtype, orange, vermilion and purple eyes. This is also demonstrated by the fact that in the eyes of vermilion sepia (v se) flies where, due to the action of the vermilion gene no early phase pigment is present, all the granules are small and yellow in colour (Plate II, fig. h.) The early phase granules in the secondary pigment cells of the sepia eye undergo a final change. During early adult life they gradually change back from red to brown or yellow so that in the sepia eye of an old adult the pigment granules are all either brown or yellow. The granules in the primary pigment cells resemble those of wildtype.

The eyes of eosin (w^e) pupae in the late phase of development are very little different from those of younger pupae (Plate II, fig. c.) A few yellowish granules are present in the secondary pigment cells of pupae 141 hours after pupation. Similar granules were observed in sections of very old vermilion-eosin adults indicating that these granules are produced very late in the pupal period and become only very slightly red.

The histological observations show that practically all of the deviations from the normal course of eye pigment development concern granules in the secondary pigment cells only. In all mutants where granules are present in the primary pigment cells they are

yellow when they appear and take on various shades of brown as they become older.

Variations in pigment distribution in the eyes of wildtype and the mutants under consideration are summarised in Table II.

Table II.

Table II.

Late Phase of Pigment Development (over 120 Hours after pupation.)

	120-132 hours after pupation	132 hours after pupation to emergence	Post pupal development
wildtype	P. yellow and brown granules S. numerous additional yellowish orange granules	P. granules darker in colour S. late phase granules increase in size and redness	P. granules very dark and coarse S. late phase granules continue to increase in size and redness
sepia (se)	P. same as wildtype S. same as wildtype	P. same as wildtype S. late phase granules remain small and yellow	P. same as wildtype S. all granules in secondary cells eventually yellow and brown
purple^{1} (pr^{1})	P. same as wildtype S. no additional granules. Early phase granules brown and yellow	P. same as wild type S. some early phase granules become red toward the end of this period	P. same as wildtype S. all early phase granules eventually red. No late phase granules.
purple^{2} (pr^{2})	P. same as wildtype S. same as wildtype	P. same as wildtype S. late phase granules less red and smaller than wildtype	P. same as wildtype S. similar to wildtype but late phase granules never become as large or as red
purple^{3} (pr^{3})	P. same as wildtype S. no late phase granules. Some red among early phase granules	P. same as wildtype S. early phase granules red. A few small yellowish late phase granules appear at this time	P. same as wildtype. S. late phase granules increase in redness but not as much as in purple^{2}.
eosin (w^{e})	P. same as wildtype S. no late phase granules present	P. same as wildtype S. a few yellowish late phase granules present	P. same as wildtype S. late phase granules coarse but few in number and light in colour.
white (w^{5})	no granules in either type of pigment cell	no granules in either type of pigment cell	no granules in either type of pigment cell
vermilion (v) orange (or)	P. no granules present S. numerous yellowish orange granules similar to late phase granules in wildtype	P. no granules present S. orange granules increase in size and redness	P. occasional granules like the ones in the secondary pigment cells S. orange granules continue toincrease in size and redness

P = primary pigment cells
S = secondary pigmentncells

DISCUSSION:

The early phase granules which appear in the eye after the 96th hour of pupal life are yellow when laid down. In the secondary pigment cells they become brown and finally red. In the primary cells, though they take on various shades of brown they never become red. In adults the granules in the primary pigment cells are coarse and dark brown in colour. The late phase granules which appear after the 120th hour of pupal life are orange in colour; small in size and yellowish at first, they gradually become more red and larger as development proceeds. These granules are present chiefly in the secondary pigment cells though in a few instances they have been observed in the primary cells as well.

Analysing the histological structure of the eye at various stages in development it is apparent that eye colour genes influence the development of one or the other or both types of pigment granules in three distinct ways:-

a. by suppressing granule formation

b. by altering the rate of pigment development

c. by changing the granules qualitatively.

a. Some eye colour genes suppress granule deposition. Orange (or) and vermilion (v) prevent the formation of all early phase granules in the primary as well as in the secondary pigment cells, but do not interfere with the deposition of the late phase granules

which appear and follow the normal course of development as in wildtype. White (w^5) entirely suppresses the deposition of both early and late phase granules. Eosin (w^e) an allele of white, does not seem to affect the granules of the primary pigment cells, but prevents the appearance of part of the early phase granules normally present in the secondary pigment cells. The few granules laid down during the early phase of development in the secondary pigment cells turn red as do similar pigment granules in wildtype. During the late phase of development very few additional granules appear. These do not become red in the late pupal period and in the adult their change of colour is very slight. The influence of the eosin (w^e) gene is very slight at the onset of pigment development (in pupae over 96 hours old) allowing the formation of all of the yellow granules in the primary pigment cells and a few of the granules in the secondary cells. This gene, however, prevents the formation of the late phase granules until the very end of the pupal period when a few of these appear but fail to develop completely.

b. <u>Some eye colour genes influence the rate of pigment development</u>. The allelomorphs of purple influence the rate of production of red pigment in the secondary pigment cells. They have no effect upon

the granules in the primary pigment cells. Purple^{2} (pr^{2}) has no visible effect on the early phase granules and does not postpone the deposition of the late phase granules, but it does retard their change from yellowish orange to red and their growth in size. Purple^{3} (pr^{3}) prevents the early phase granules from becoming red until after the 120th hour of pupal life. The change to red is very slow and is not complete until nearly the end of the pupal period. The late phase granules, when they appear toward the end of the pupal period, remain smaller than the corresponding granules in purple^{2} (pr^{2}) pupae and their rate of reddening is very much slower. Purple^{1} (pr^{1}) prevents the transition from brown to red in the early phase granules until the very end of pupal life, and retards it to such an extent that only in the old adult is the change to red complete. No late phase granules ever appear in purple^{1} eyes. Text fig. 1. shows graphically how these three allelomorphs of purple affect pigment development as compared with wildtype, and how their different rates of development influence the final colour of the eyes.

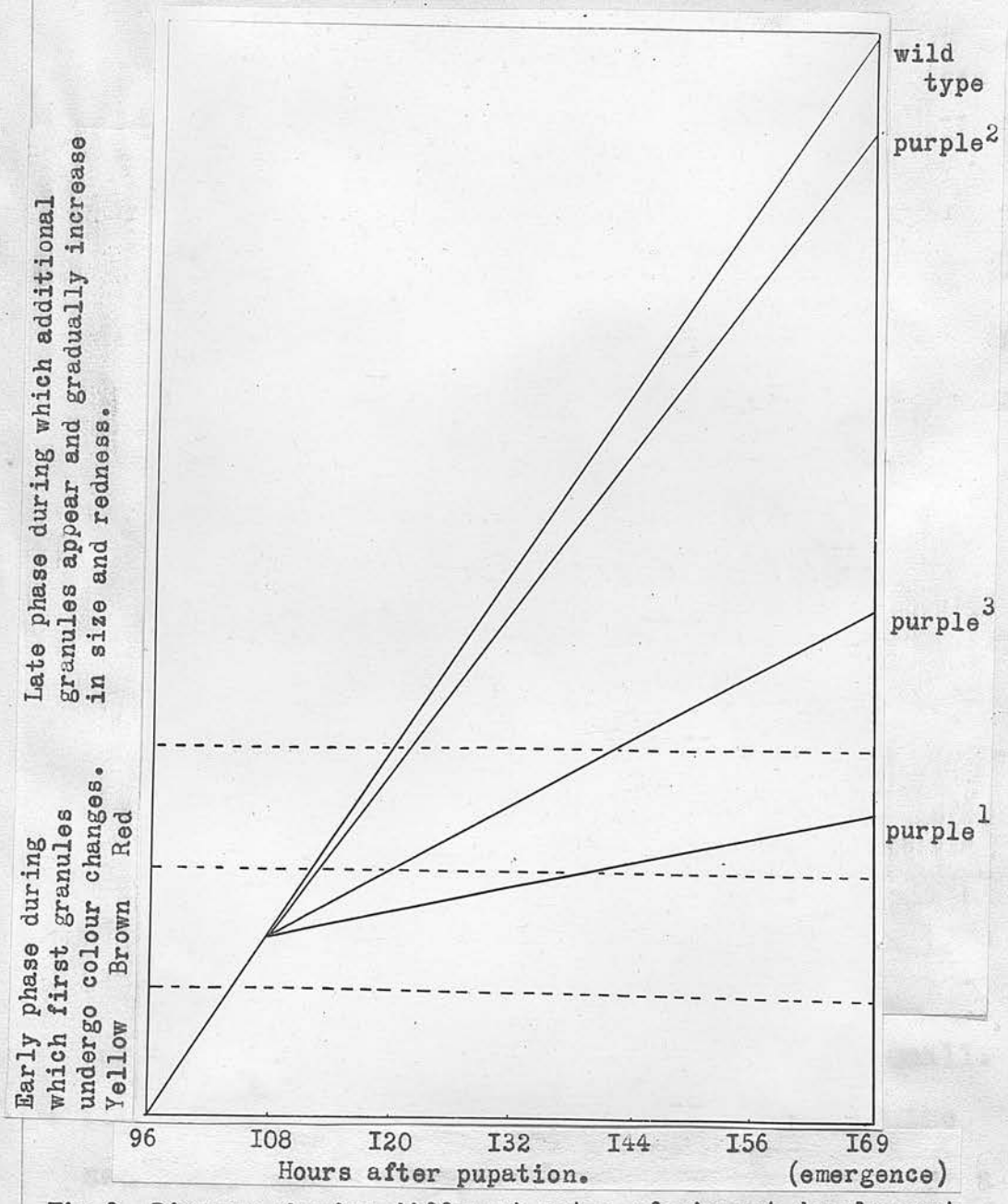

Fig.1. Diagram showing different rates of pigment development in the presence of three purple allelomorphs and wild type of D. pseudo-obscura.

It is apparent that the three allelomorphs of purple influence pigment production in the same manner but in different degrees. The differences are what Goldschmidt (1927) has called quantitative differences of the allelomorphs acting on one and the same process. Purple^{1} may be classified as the strongest allele because it retards the process of

pigment development most. Purple^{3} (pr^{3}) has much less effect, and purple^{2} has so little effect as to make the eye barely distinguishable from wildtype. Ford and Huxley (1928) and Wolsky and Huxley (1933) have described a similar situation in Gammarus chevreux; where an allelomorphic series of genes has varying effects on eye pigment due to varying the rates and extent of pigment deposition.

c. Some eye colour genes affect the pigment qualitatively. Sepia eyes are identical with wildtype until the end of the early phase of pigment development. At this time the primary pigment cells contain only yellow and brown granules and the secondary cells only red ones. The usual number of late phase granules are formed but instead of becoming red and large in size they remain yellow and small. During adult life the early phase granules in the secondary cells which were previously red undergo a reversed chemical reaction and become yellow. Text fig. 2 illustrates how the proportions of red to yellow and brown pigment granules in the secondary pigment cells of wildtype and sepia eyes differ at different times during development.

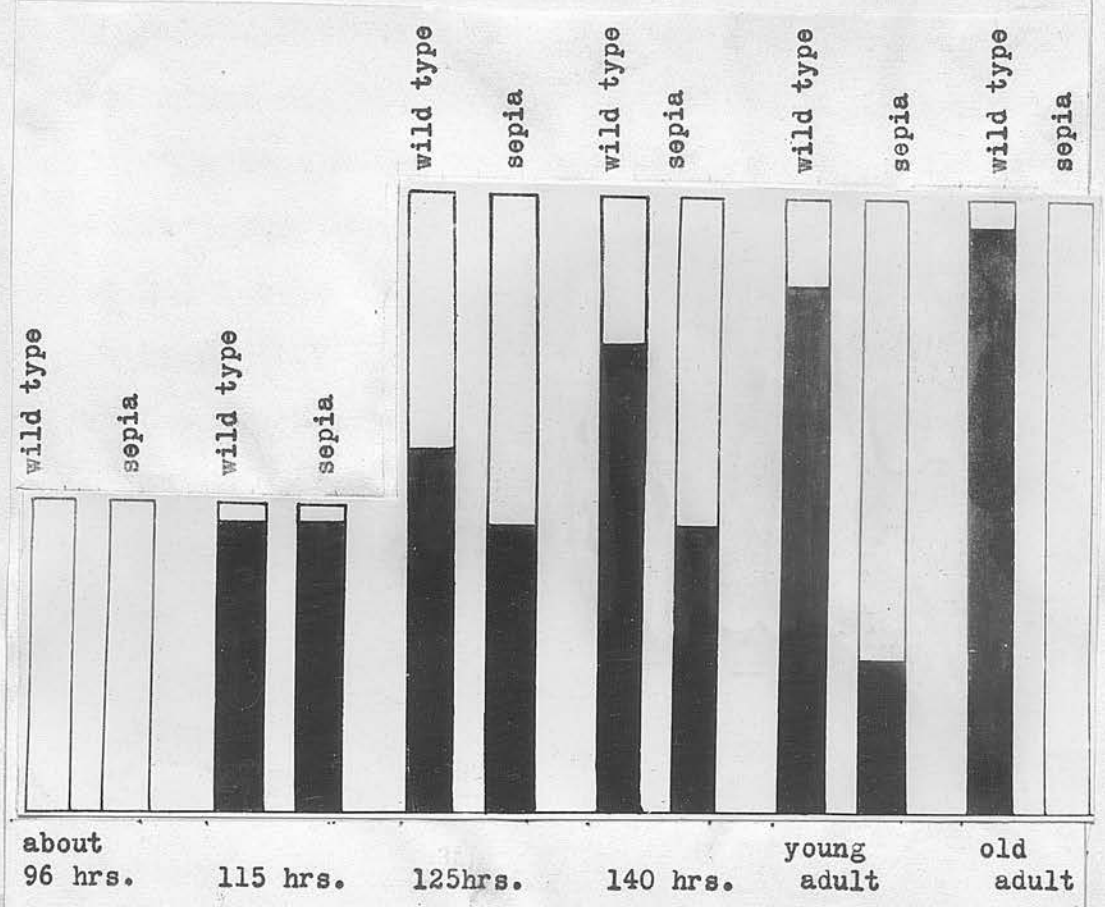

Fig. 2. Diagram showing the proportion of red to total pigment in the eyes of wild type and sepia flies at various stages of development. Shaded portions of lines indicate the amount of red.

According to Schultz (1935) yellow pigment may be an oxidation product of red. It is probable, as suggested by Mainx (1935) that the sepia gene eliminates the inhibition of oxidation of red pigment which is characteristic of the wildtype allele of sepia. As a result of this elimination all the granules in the secondary cells of the sepia eye may be oxidised to become yellow. As yellow pigment readily changes to brown, it is natural that brown granules should appear among the yellow ones in the

secondary pigment cells. Bridges (1919) in D. melanogaster, and Crew and Lamy (1935 in D. pseudo-obscura have noted that old sepia flies have much darker eyes than wildtype. This very dark colour is not due to the presence of more pigment granules; it is obvious that the numerous coarse red granules in the secondary pigment cells of the wildtype eye interfere with the distinct observation of the brown colour of the granules in the primary pigment cells, while the yellow and brown granules in the secondary cells of the sepia eye intensify the effect of the brown granules in the primary cells of the sepia eye.

The detailed analysis of the histological structure of the eye during the various stages of its development clearly indicates that the maximum amount of pigment is present in the eyes of wildtype flies. It also shows how mutant genes alter the process of pigment formation and behaviour. Each gene comes into operation at a definite stage and remains effective during a limited period of time. The affect is specific for each gene.

SUMMARY:

A histological study of wildtype and seven eye colour mutants of Drosophila pseudo-obscura at various stages of development has been made. The genes concerned may be classified according to their action, so:-

1. Genes which suppress pigment formation: a. Vermilion (v) and orange (or) which suppress the early phase of development entirely but allow the late phase to proceed as in wildtype. b. White (w^5) and eosin (w^e) which suppress granule formation. The suppression is complete in the case of white, partial in eosin. Eosin does not affect the subsequent development of the granules which do appear.

2. Genes which alter the rate of pigment production The three allelomorphs of purple (pr) which affect the rates of production of red pigment in varying degrees. Purple^{2} (pr^{2}) retards the development of red so little as to make the eye almost indistinguishable from wildtype. Purple^{3} (pr^{3}) retards the development of red considerably, and purple^{1} (pr^{1}) to such an extent that few red granules are present at emergence though many appear in older flies.

3. Genes which have a qualititative affect upon pigment. Sepia (se) influences the development of pigment during the later stages of pupal life and early adult life. All of the eye pigment of sepia flies eventually becomes yellow and brown, which indicates that the influence of sepia is qualitative and that the normal amount of pigment is probably unchanged.

Description of Plates.

Semi-diagramatic longitudinal sections of omatidia of the eyes of D. pseudo-obscura, showing the distribution of pigment granules at various stages of development.

Plate I.

Fig. a. vermilion (v), orange (or) or white (w^5) pupa about 104 hours old.

b. wildtype, sepia (se), purple (1,2 or 3) about 104 hours old.

c. eosin (w^e) pupa about 104 hours old.

d. purple^1 (pr^1) or purple^3 (pr^3) pupa 115-120 hours old.

e. wildtype, sepia (se) or purple^2 (pr^2) pupa 115-120 hours old

f. eosin (w^e) pupa 115-120 hours old

g. orange (or) or vermilion (v) pupa 120-132 hours old.

h. wildtype pupa 120-132 hours old.

i. purple^3 (pr^3) pupa 120-132 hours old.

Plate II.

Fig. a. vermilion (v) or orange (or) pupa about 140 hours old.

b. wildtype pupa about 140 hours old

c. eosin (w^e) pupa about 140 hours old

d. sepia (se) pupa about 140 hours old.

e. purple^{1} (pr^{1}) pupa about 140 hours old

f. purple^{2} (pr^{2}) pupa about 140 hours old

g. purple^{3} (pr3) pupa about 140 hours old

h. vermilion sepia (v se) adult

i. orange (or) or vermilion (v) adult about two weeks old.

j. orange purple^{2} (or pr^{2}) adult about two weeks old

k. orange purple^{3} (or pr^{3}) adult about two weeks old.

l. sepia (se) adult about two weeks old.

Explanation of lettering.

c	-	cornea
pcc	-	pseudocone cell
ppc	-	primary pigment cell
spc	-	secondary pigment cell
r	-	retinula
rh	-	rhabdomere
bm	-	basement membrane.

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Three new Eye-colour Mutations in Drosophila pseudo-obscura.

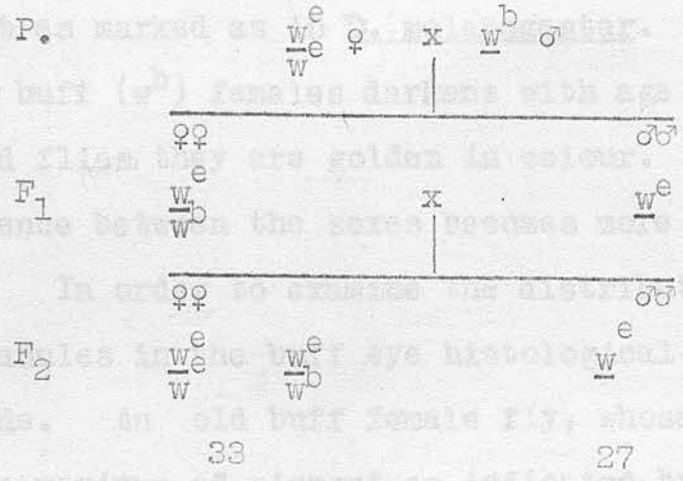
Three new Eye-colour Mutations in Drosophila pseudo-obscura.

The present paper describes three new recessive mutations affecting eye-colour. The role of these mutant genes was investigated by studying pigment formation during development and by analysing their effects in various combinations with other genes which influence the production of eye-colour.

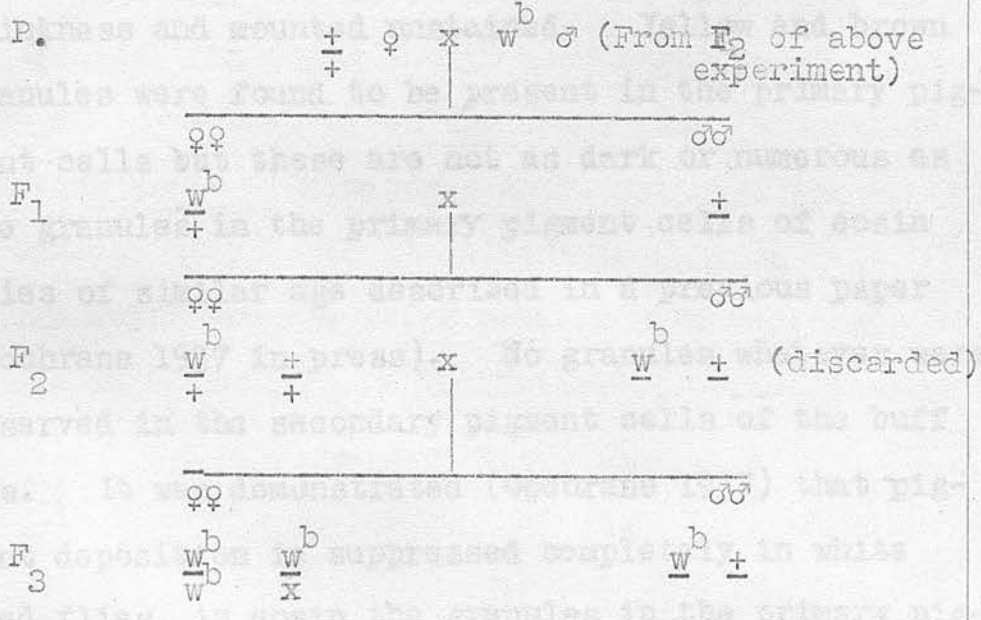
i. buff (w^b)

In April 1936, among the offspring from a brother sister pair mating of eosin (w^e) flies there appeared three males with an eye colour very much lighter than that of the other flies in the culture. These were called "buff" because of their similarity to buff of D. melanogaster, first reported by Safir (1916) and included by Morgan, Bridges and Sturtevant in the list of mutant characters in "Genetics of Drosophila" (1925) There, buff, an allele of white, is described as an eye colour pale pinkish yellow, lighter than eosin male. To test whether this new eye colour mutation was also the expression of a white allelomorph the original buff eyed males were mated to eosin (w^e) females from the same culture. The females in the F2 generation had eyes similar to but slightly lighter in colour than usual for eosin (w^e). This shows that the new recessive eye colour (buff) is the expression of an allele of eosin (w^e) a sex linked recessive gene (described by Lancefield (1922) and found to be an allele of white. It was given the symbol (w^b).

To determine the genetic behaviour of the new mutant the following breeding test was made.



In order to build up a homozygous buff (w^b) stock the following crosses were made:



The eyes of the buff (w^b) females have a deeper colour than the eyes of the males. (See Plate I. figs. A. & B.) This sexual dimorphism is characteristic of white allelomorphs in D. melanogaster

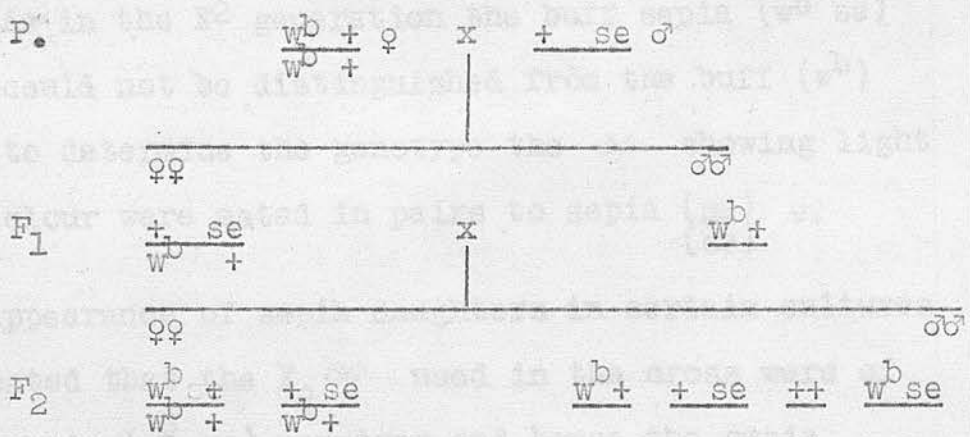
(Morgan & Bridges 1913) as well as in D. pseudo-obscura. In eosin (w^e) of D. pseudo-obscura it is not as marked as in D. melanogaster. The eye colour of buff (w^b) females darkens with age until in very old flies they are golden in colour. Thus the difference between the sexes becomes more pronounced.

In order to examine the distribution of pigment granules in the buff eye histological sections were made. An old buff female fly, whose eyes contained the maximum of pigment as indicated by the intensity of colour, was selected, fixed in Carnoy Lebrun, and embedded by the quick cellodin-paraffin method of Peterfy (1928). Sections were cut 7 microns in thickness and mounted unstained. Yellow and brown granules were found to be present in the primary pigment cells but these are not as dark or numerous as the granules in the primary pigment cells of eosin flies of similar age described in a previous paper (Cochrane 1937 in press). No granules whatever were observed in the secondary pigment cells of the buff eye. It was demonstrated (Cochrane 1937) that pigment deposition is suppressed completely in white eyed flies, in eosin the granules in the primary pigment cells are unaffected and a few early and late phase granules are present in the secondary pigment cells. Comparison of the histological structure of the eyes of these mutants indicates that the buff (w^b) gene has a greater effect upon eye pigment

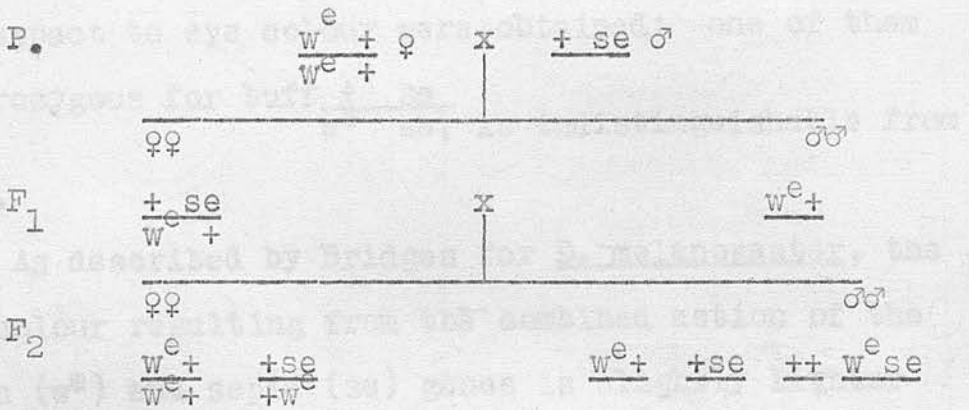
development than eosin (w^e) but a smaller effect than white (w^5). Buff (w^b) therefore stands between its two allelomorphs, white (w^5) and eosin (w^e) in order of effectiveness in suppressing pigment deposition.

Bridges (1919) investigating the specific modifyers of eosin eye colour in D. melanogaster made the interesting observation that sepia (se) and eosin (w^e), two sex linked but non allelomorphic genes, in combination, produce an even lighter eye colour than eosin alone. He classified sepia (se) as a reversed modifyer of eosin (w^e) on the assumption that although sepia eyes contain much more colour than wildtype, the sepia (se) gene is capable of affecting eosin (w^e) in such a way as to produce an eye lighter than eosin rather than darker one. Because of the behaviour of the sepia gene in combination with white allelomorphs in D. melanogaster it was decided to analyse its effect in D. pseudoobscura using the new white allelomorph, buff (w^b) as well as eosin (w^e). The following crossings were made:

To obtain buff-sepia cross-overs:



To obtain eosin-sepia cross-overs:



As in the F^2 generation the buff sepia (w^b se) ♂♂ could not be distinguished from the buff (w^b) ♂♂, to determine the genotype the ♂♂ showing light eye colour were mated in pairs to sepia $\left(\frac{se}{se}\right)$ ♀♀ The appearance of sepia daughters in certain cultures indicated that the F_2 ♂♂ used in the cross were of buff sepia (w^b se) genotype and hence the sepia daughters were heterozygous for buss (w^b). These heterozygous ($\frac{+_b\ se}{w\ se}$) ♀♀ were mated to their sepia brothers and their daughters mated to buff sepia (w^b se) sons. From this mating two kinds of ♀♀ in respect to eye colour were obtained: one of them heterozygous for buff $\frac{+\ \ se}{w^b\ \ se}$, is indistinguishable from buff.

As described by Bridges for <u>D. melanogaster</u>, the eye-colour resulting from the combined action of the eosin (w^e) and sepia (se) genes is slightly lighter than that of eosin. The interaction of the sepia (se) and eosin (w^e) genes and that of sepia (se) and buff (w^b) in <u>D. pseudo-obscura</u>, can be explained in the light of present knowledge of pigment development of these mutants (Cochrane 1937). In eosin very few early as well as late phase granules are present in the secondary cells. The early phase granules become red; the late phase granules become coarse and reddish in color. The specific function of the sepia (se) is to affect both late and early phase granules

in the secondary pigment cells. The former remain small in size and yellow in colour while the latter gradually become yellow and brown. The few granules which eosin permits to develop in the secondary pigment cells behave in the presence of the sepia (se) gene as corresponding granules do in sepia eyes, i.e. they remain yellow and hence the eye colour in young flies appears lighter than eosin.

Histological sections of a young eosin-sepia (w^e-se) fly show practically no differences from the eosin eye. Possibly there is a larger proportion of yellow granules in the secondary pigment cells but the total number of granules is so small that the difference could not be exactly determined.

In old eosin-sepia (w^e-se) flies the eyes are dull brown in colour whereas in eosin (w^e) flies of the same age they are reddish brown. This behaviour is the typical result of the action of the sepia (se) gene which causes eye-colour to become very dark during adult life. (Crew and Lamy 1935) and (Morgan, Bridges and Sturtevant 1925). In buff eyes there are no granules in the secondary pigment cells, and because sepia (se) does not affect the granules of the primary pigment cells, the eye-colour of buff-sepia (w^b)-se) flies is indistinguishable from buff.

ii. rust^1

Source of rust^1. In a mass culture of scutellar-yellow vermilion (sc-y-v) flies a few males were found whose eyes were much lighter in colour than typical vermilion eyes. These were mated to wildtype females. In the F_2 generation there were three classes of males, having respectively the following eye-colours: vermilion, the new light vermilion and a new colour. This showed that the light vermilion eyed males were the expression of a new sex linked recessive mutant acting in combination with vermilion. This mutant was called rust^1 (rst^1).

Description of rust^1. Rust^1 eyes are similar in colour to wildtype but readily distinguishable. They are more transparent than wildtype, duller in tone than vermilion and less brown than any of the purple allelomorphs. (Plate I. Fig C.) The testis sheaths while less coloured than those of wildtype (+) orange (or) or vermilion (v) flies is distinctly orange in colour.

Genetics of rust^1. In order to test rust^1 (rst^1) for allelomorphism with other sex linked eye-colour genes, rust^1 (rst^1) ♂♂ were mated to sepia (se) ♀♀ eosin (w^e) ♀♀ and buff (w^b) ♀♀ respectively. From these crosses only phenotypically wildtype (+) ♀♀

appeared in the F_1 generations showing that rust^1 (rst^1) is not allelomorphic to sepia (se) or white (w^5). In order to determine the genetic locus of rust^1 (rst^1) in relation to sepia (se) the F_1 ♀♀ from the $\frac{se}{se}$ ♀ x $\frac{rst^1}{}$ ♂ cross were mated in pairs to wildtype males. Counts of their male offspring are given in Table I.

Table I. Classes of F_2 ♂♂ from se ♀ x rst ♂ cross.

Culture No.	non cross-over classes		cross over classes		Total
	sepia	rust	wildtype	sepia-rust	
I.	17	18	2	2	39
II.	19	25	1	7	52
III.	18	18	3	5	44
IV.	15	17	4	7	43
V.	19	13	7	4	43
VI.	17	22	5	7	51
VII.	7	14	5	6	32
VIII.	16	13	2	2	33
IX.	11	19	4	4	38
X.	14	17	4	0	35
XI.	8	15	3	1	27
XII.	16	11	7	5	39
XIII.	14	17	4	3	38
XIV.	16	10	3	4	33
XV.	13	18	4	3	38
XVI.	17	8	3	6	34
XVII.	20	6	3	3	32
XVIII.	15	19	1	2	37
Total	272	283	65	71	688

Total flies showing crossing over between rust and sepia 136 or approximately 20%

These data indicate that the map distance between rust (rst^1) and sepia (se) is approximately 20 cross-over units.

To determine the position on the chromosome map more exactly,

a second cross was made between a yellow vermilion rust female and a sepia male. The F_1 females were out crossed to wildtype males in pairs. Table II. gives the numbers of the various classes of males resulting from this cross.

This shows 28.4% crossing over between vermilion and rust and about 20% between rust and sepia. Hence the locus of rust is between vermilion and sepia about 28 units to the right of vermilion and 20 to the left of sepia.

Cross:- $\frac{y\ v\ rust^{1}\ +}{+\ +\ +\ se}$ ♀ x $\frac{+\ +}{}$ ♂ Table II.

Culture numbers	♀♀	yv rust	sepia	y sepia	v rust	yv sepia	rust	yv rust se	wild	y rust	v se	y	v rust se	yv	rust se	Total ♂♂
I.	70	17	17	1	1	7	11	6	10	1	0	0	2	1	1	75
II.	53	12	19	2	1	7	13	2	4	1	0	0	0	1	4	68
III.	78	22	22	1	3	3	9	4	5	0	0	0	0	3	2	78
IV.	69	14	21	0	2	5	8	8	2	0	0	0	2	0	1	62
V.	82	17	20	2	2	12	12	5	3	1	0	0	0	1	0	75
VI.	91	9	19	0	3	3	6	3	6	1	0	0	1	1	1	53
VII.	15	0	3	1	0	2	1	0	0	0	0	0	0	1	1	9
VIII.	75	18	19	0	2	6	7	5	6	7	1	2	0	1	0	74
IX.	74	11	8	0	2	2	7	7	10	0	0	2	1	0	2	53
X.	97	26	13	2	1	5	12	2	5	0	0	0	1	0	1	68
XI.	89	15	20	2	1	8	11	5	5	2	1	0	0	2	1	73
XII.	109	19	16	1	1	9	9	6	3	0	0	0	1	2	1	68
XIII.	75	21	20	3	2	8	8	4	6	1	0	0	2	0	2	77
XIV.	99	16	14	1	3	10	15	2	11	4	0	0	1	1	1	80
XV.	110	29	25	0	2	9	7	12	7	1	0	1	0	0	1	94
XVI.	55	15	15	1	1	3	10	7	6	2	0	0	0	1	3	58
Total	1241	261	272	17	27	99	146	78	89	18	2	5	9	16	22	1065.

Cross overs:-

Regions	1	2	3	1,2	1,3	2,3	1,2,3
	44	245	167	20	14	38	none

% recombination:

y-v, 7.3; v-rst^{1}, 28.4; rst^{1}-se, 20.5;

Development of eye-colour in rust[1]. Pupae of rust flies were isolated during the first hour after pupation, incubated at 25° C for periods of varying lengths and dissected under a Zeiss binocular microscope. The times found to be important in the eye pigment development of wildtype and other eye-colour mutants (Cochrane 1936) were taken as a guide for this work and rust[1] pupae were dissected after incubation for the same periods. Table III. includes all the data obtained from these dissections and gives in addition data obtained from dissections of wildtype pupae of similar ages for comparison.

Table III. Dissection records of rust[1] pupae describing eye-colour in various stages of development.

Age of pupae.	No. of pupae	Colour of eyes/ rust[1]	wildtype	Incidental Data
96-108 hrs.	5	yellow → tan	yellow → tan	
109-120 hrs.	17	tan→pink	tan → red	testes pink
121-132 hrs.	8	orange (dilute)	orange (opaque)	testes pink
132-	11	brownish orange (Lighter than wild-type.)	orange-brownish red.	testes orange

These dissection records show that in the rust eye the sequence of events in pigment development is the same as in wildtype. The first pigment appears

at about the same time as in wildtype pupae. It is yellow in colour when it appears and changes to tan and pink during the early phase of development. During the late phase the colour becomes more opaque and orange. The difference from wildtype lies in the fact that at every stage of development rust eyes are lighter in colour suggesting that the rust^{1} (rst^{1}) gene is one which acts throughout the entire period of pigment development to prevent the deposition of a part of the pigment granules normally laid down without altering the normal sequence of events or producing any qualititative differences.

Histology of rust^{1} eyes. A study of sections of pupal eyes at various ages revealed the complete absence of granules in the primary pigment cells of rust^{1} eyes. It is also clear that very few granules are laid down in the secondary pigment cells during the early phase of pigment development. In pupae 112 hours old these few granules are brown and yellow. In pupae a few hours older the number of granules is the same but instead of being brown and yellow they are red. In still older pupae the secondary pigment cells contain numerous orange granules similar to, but fewer in number than, the orange granules which appear in the secondary pigment cells of wildtype eyes during the late phase of development. These data afford evidence that the rust^{1} (rst^{1}) gene affects the

deposition of pigment granules. The affect is very marked in the early phase of development but less so in the late phase.

Rust^{1} in combination with other mutant eye-colour genes.

a. vermilion (See Plate I. Fig. D.) The eye produced by the combined action of vermilion (v) and rust^{1} (rst^{1}) is lighter in colour than vermilion. The dilution is similar to that produced by purple^{2} (pr^{2}) in combination with vermilion. (Crew and Lamy 1934). The testis colour is similarly affected. Such behaviour can be explained by the fact that while vermilion (v) suppresses the early phase granules rust must affect the granules deposited during the late phase of development. The power of the rust^{1} gene in suppressing late phase development may be measured by comparing the vermilion-rust^{1} eye with the vermilion eye.

b. sepia. (See Plate I. Fig E.) In combination with sepia (se), rust^{1} (rst^{1}) produces an eye similar in colour to the rust eye but somewhat duller in tone and more brown. This is due to the fact that while rust^{1} (rst^{1}) acts quantitatively to prevent the formation of many of the granules present in the wildtype eye, sepia gradually changes the colour of the ones which remain to yellow. Hence the rust^{1}-sepia eye is duller than the rust1 eye due to the absence of part of the red granules.

c. eosin. (See Plate I. Fig. F.) In the F_2 generation of the eosin (w^e) x rst^1 cross several males were present whose eyes were lemon in colour. In order to test the supposition that these lemon-eyed males were eosin-$rust^1$ cross-overs they were mated in pairs to both $rust^1$ (rst^1) and eosin (w^e) females. All the female offspring of the $rust^1$ mothers had rust eye-colour indicating that $rust^1$ (rst^1) was present in the genetic constitution of the lemon eyed flies. Similarly, all the female offspring of the eosin mothers had eosin eye-colour showing that eosin (w^e) was also a genetic constituent of the lemon eyed flies. The eyes of the eosin-$rust^1$ fly are very slightly coloured which indicated that eosin and rust must suppress pigment formation during different periods of time and that between them they must suppress nearly all deposition of pigment. From previous histological studies (Cochrane 1937 in press) it was ascertained that eosin (w^e) allows all of the granules normally present in the primary pigment cells to appear but prevents the deposition of a large number of the granules normally formed in the secondary pigment cells during the early phase of development and nearly all of those laid down during the late phase. $Rust^1$ suppresses all of the granules normally present in the primary pigment cells. It also suppresses nearly all of the early phase granules and part of the

late phase granules normally present in the secondary pigment cells.

The eosin-rust[1] eye is the expression of the combined action of two genes each of which prevent the deposition of part of the granules normally present in the wildtype eye. As their combined affects involve nearly all of the granules a very light eyed fly is the result. Fig. I shows graphically how the rust[1] gene affects the eye pigment when acting alone and in combination with other genes.

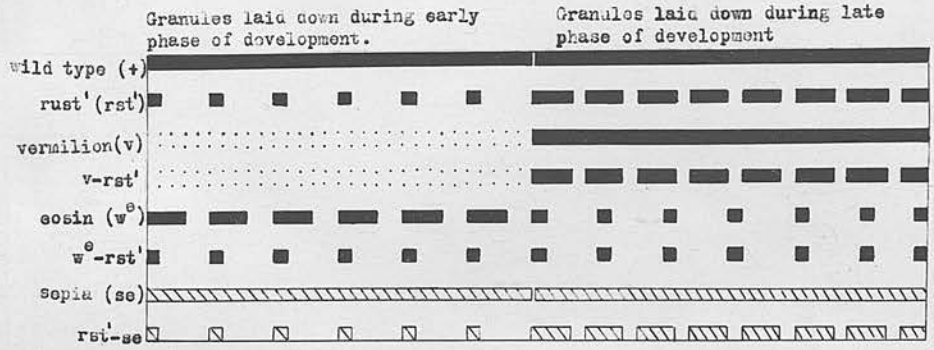


Fig. I. Diagram to show the relative quantities of pigment granules laid down in the eyes of rust[1] and other eye-colour mutants of D. pseudo-obscura during each phase of pigment development. Dotted lines indicate total absence of granules. Broken lines indicate a reduced number of granules as compared with wildtype, the amount of black indicating the amount of pigment formed. Shaded lines indicated a colour difference brought about by the action of the sepia (se) gene.

iii rust^2 (rst^2)

Among the F_2 male offspring of the eosin (w^e) x rust^1 (rst^1) cross there appeared a few males whose eyes resembled those of eosin (w^e) but whose testes were like those of rust^1 flies. These were mated to rust^1 ♀♀ and to eosin ♀♀ at the same time. The eosin ♀♀ did not survive but the F_1 offspring of the rust^1 mothers were all rust indicating that rust was a genetic constituent of the original flies. In the F_2 generation there were: rust ♂♂ and ♀♀ the new light rust ♂♂ and some light eyed ♀♀ which were thought to be heterozygous for the new eye-colour. The new eye-colour gene was called rust^2 (rst^2) for it was found to be an allele of rust^1. In the F_3 generation there were both ♂ and ♀ rust^2 flies from which a stock was made up. The eyes of rust^2 flies are only slightly lighter and more yellow than rust^1 and the testes are indistinguishable. For the colour of the eyes see Plate I. Fig G.

The behaviour of the rust^2 (rst^2) gene in combination with other eye colour genes is consistant. With vermilion (v) an eye slightly lighter in colour and yellower than the rust^1-vermilion eye is produced. See Plate I. Fig H. Similarly in combination with the sepia (se) gene an eye lighter in colour than the rust^1-sepia eye is produced. See Plate I. Fig. K.

Summary.

1. Three new eye-colour mutants of *D. pseudo-obscura* are described and named: buff, rust^1 and rust^2.

2. The genetic loci of all three have been ascertained by breeding experiments. They were all found to be sex linked. Buff is an allele of white (w^5) and eosin (w^e) and has been given the symbol w^b. Rust^1 and rust^2 have been found to be allelomorphic to each other and are located 28 crossover units to the right of vermilion (v) and 20 to the left of sepia (se) on the x chromosome.

3. The combined effects of these with other eye-colour genes are described.

4. The development and histology of rust^1 have been studied in detail and compared with wildtype.

Description of Plate I.

A. buff (w^b) ♂ showing eye colour.

B. buff (w^b) ♀

C. $rust^1$ (rst^1) ♂

D. vermilion-rust (v-rst) ♂

E. rust-sepia (rst-se) ♂

F. rust-eosin (rst-w^e)

G. $rust^2$ (rst^2)

H. vermilion-rust (v-$rust^2$)

K. $rust^2$-sepia (rst^2-se)

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Genetic and Developmental Relationships of Testis and Eye-colour in *Drosophila pseudo-obscura*.

Genetic and Developmental Relationships of Testis and Eye-colour in Drosophila pseudo-obscura.

Introduction.

The fact that the colour of the testis sheath in Drosophila is in some way connected with eye-colour was pointed out by Dobzhansky (1927). He made several genetic experiments using wildtype and various eye-colour mutants of D. melanogaster which showed that testis colour and eye-colour are invariably associated. Any fly having a wildtype eye had also yellow testis colour (the colour which is characteristic of wildtype in D. melanogaster.) any fly having colourless eyes, regardless of the genetic constitution, had also colourless testes. Such an association may be explained by assuming that it is due either to closely linked genes or to the action of one and the same gene acting simultaneously upon two differend organs.

Studies by Dobzhansky(1931) and Sturtevant (1932 on gynandromorphs of D. simulans show that eye pigment and testis pigment must arise from a common "precurser" present in all wildtype tissue though the pigment when it appears has two different forms, granular in the eye and diffuse in the testes.

In the course of work on eye pigment development (Cochrane 1936) and the genetics of new mutant eye colours (1937 unpublished) in D. pseudo-obscura considerable incidental data on testis colour were recorded. These data seemed so important that a comparative study of the testis colour in the various

various eye-colour mutants was undertaken.

Material and Methods.

Adult males within twenty four hours after emergence were selected from the following stocks:

Wild type (+)	vermilion-purple^1 (v-pr^1)
orange (or)	vermilion-purple^2 (v-pr^2)
vermilion (v)	orange-purple^2 (or-pr^2)
sepia (se)	Orange-purple^3 (pr-pr3)
eosin (w^e)	vermilion-sepia (v-se)
purple^1 (pr^1)	rust-sepia (rst-se)
purple^2 (pr^2)	eosin-rust (w^e-rst)
purple^3 (pr^3)	eosin-sepia (w^e-se)
rust (rst)	buff-sepia (w^b-se)
buff (w^b)	vermilion-rust (v-rst)
white^5 (w^5)	

At least twenty males from each stock were used. These were isolated in separate vials for two days in order to allow for the completion of pigment formation in eyes and testes. Testes were dissected out in normal saline solution and examined under a Zeiss binocular microscope using an electric lamp and a copper sulphate solution filter. The colour differences were so readily observed that no complicated method of measuring colour was required.

In order to ascertain the time at which pigment appears in the testis sheath and to determine whether there is any correlation between that time and the times of eye pigment deposition, pupae were isolated within the first hour after pupation, incubated at 25°C and dissected after definite periods of time. Both testis and eye colour of the same pupae were examined under the dissecting microscope using the same artificial light that was used for examining the adult testes.

Description.

In D. pseudo-obscura the testes are paired oval bodies connected to the vas deferens by the vasa efferentia. See Plate I. In wildtype and many eye-colour mutants the testes sheaths and vasa efferentia are orange coloured. In various other mutants they are colourless and in sepia and in combinations involving sepia they are yellow.

Testis colour in the various mutants under consideration is described in Table I. and illustrated in Plate I.

Table I.

Type	Testis colour in adults.
wildtype ( )	orange.
vermilion (v) and orange (or)	" , slightly lighter than orange in wildtype.
purple^2 (pr^2) v-pr^2 or-pr^2	" , more dilute than colour in orange and vermilion.
purple^3 (pr^3) or-pr^3	" , very dilute in testes but darker in vasa efferentia.
purple^1 (pr^1) v-pr^1	colourless.
rust (rst) v-rst	similar to purple^2.
eosin (w^e)	similar to purple^3, a little deeper colour.
w^e-rst	orange, lighter than purple3.
sepia (se) v-se	yellow.
rst-se	" , dilute.
w^e-se	" , very dilute.
white (w^5) buff (w^b) buff-sepia (w^b-se)	colourless.

Mutants which have orange testes may be arranged in a series according to the quantities of colour present: wildtype; orange and vermilion; purple^{2}, orange-purple^{2}, vermilion-purple^{2}, rust and vermilion-rust; eosin; purple^{3} and orange-purple^{3}; and eosin-rust. A similar series of flies with yellow testes would include sepia and vermilion-sepia; rust-sepia and eosin-sepia. A third group of flies with colourless testes includes purple^{1}; vermilion-purple^{1}, white, buff and buff-sepia.

Examination of pupae of various ages has shown that colour appears in the testes of wildtype about one hundred and sixteen hours after pupation. Comparing this with the times of pigment deposition in the eyes it is seen that pigment appears in the testis after the deposition of early phase eye-pigment (Cochrane 1936) and before the onset of the late phase. The testes of orange pupae of 118 hours are coloured, while the eyes of this mutant are still colourless due to the absence of early phase pigment. As it is known (Cochrane 1936) that the late phase of eye-colour formation (coinciding with the first appearance of colour in the eyes of orange and vermilion flies) takes place at about 120 hours, this observation indicates that pigment deposition in the testes must precede by a short interval the late phase of eye-pigment formation.

In sepia and vermilion-sepia flies, colour appears in the testes a little later than in wildtype and differs in that it is yellow and remains yellow throughout development.

Testis colour does not change during the course of development though it becomes deeper with age. In all mutants showing colour dilutions in the testes of adults, proportional dilutions of colour are distinguishable in the pupae at the time of pigment deposition. Histological sections show that testis pigment in D. pseudo-obscura differs from that of other species in which it has been investigated in being granular in form and apparently similar to the eye-pigment.

Discussion.

If the quantities of pigment present in the various types of testes are compared with the total quantities of pigment in the eyes of adults of the same types, no connection is apparent. Purple[1] eyes are very dark in colour but the testes of these flies are colourless. Orange and vermilion flies have nearly as much colour in their testes as wildtype but very much less colour in their eyes. A correlation becomes apparent when the times of deposition of pigment in eyes and testes are compared. From recent studies on eye-colour (Cochrane 1936 and other unpublished work) it has been shown that

in D. pseudo-obscura there are two consecutive but independent phases of eye pigment development. Observations on the development of testis colour show that while the early phase pigment is being laid down in the eyes the testes remain colourless and that only a few hours before the onset of late phase pigment deposition in the eyes does any colour appear in the testes. This suggests that the development of colour in the testes is associated with the late phase of pigment development in the eye and not with the early phase. It might also be expected that the quantities of pigment in the adult testes and the quantities of late phase pigment present in the eyes would be comparable. Table II. shows the relative quantities of testis colour early and late phase eye pigment in the various mutants under consideration. (See Page 74)

In those eye-colours in which pigment is reduced or suppressed only in the early phase, namely vermilion and orange, there is little or no reduction in the testis colour whereas in every case where there is dilution of late phase eye-colour a proportionate dilution in the colour of the testes occurs. See Plate I. The relative dilutions of late phase eye pigment can be estimated from the amount of eye-colour present in flies in which the early phase eye pigment has been suppressed by introducing vermilion (v) or orange (or) genes. This affect of vermilion (v) and orange (or) has been described (Cochrane 1936 and 1937 in press.)

Table II. Relative Quantities of pigment in the testes and eyes of various mutants.

Eye-colour type	Testis pigment	Eye-pigment. Early phase	Eye-pigment. Late phase
Wildtype (+)	xxxxx (orange)	xxxx	xxxxx
orange (or vermilion (v)	xxxx "	-	xxxx
purple^{2} (pr^{2})	xxx "	xxxx	xxx
v-pr^{2} or-pr^{2}	xxx "	-	xxx
purple^{3} (pr^{3})	x "	xxxx	x
or-pr^{3}	x "	-	x
purple^{1} (pr^{1})	-	xxxx	-
v-pr^{1}	-	-	-
eosin (w^{e})	xx "	xxx	x-xx
rust (rst)	xxx "	xx	xxx
v-rst	xxx "	-	xxx
w^{e}-rst	x "	X	x
sepia (se)	xxxx yellow	xxxx	xxxx
v-se	xxxx "	-	xxxx
rst-se	xxx "	xx	xxx
w^{e}-se	x "	xxx	x
buff (w^{b})	-	x	-
white (w^{5})	-	-	-

Whenever sepia is present, there is a qualitative difference in the colour of the testis sheath comparable to the difference in the eye-colour. Instead of showing a specific dilution of the wildtype colour (orange) the testis sheaths of all flies with sepia (se) in their genetic constitutions are yellow. The amount of pigment is proportional to the quantity of late phase pigment in the eye. Thus an eosin-sepia fly has very little late phase eye pigment due to the action of the eosin gene and as a result of the action of the sepia gene, that small quantity of pigment is yellow. The testis pigment is affected in exactly the same way by the combined action of the two genes; one partially suppressing its development and the other causing a change in its colour.

Conclusions.

It is obvious that only those genes which affect the late phase of eye pigment development have any effect upon testis colour. This is because, in the testes, colour appears after all of the early phase eye colour has been deposited - too late for genes influencing early phase eye pigment development to have any influence.

Genes which affect late phase eye pigment affect testis pigment in exactly the same way. Those which suppress its formation entirely or in part suppress the formation of testis pigment to the same extent and those which have a qualitative effect on eye pigment have a similar effect on testis pigment.

Summary.

1. Testes of adults and pupae of wildtype and various eye-colour mutant types of D. pseudo-obscura have been examined.

2. Histological sections show that pigment is of a granular nature in the testes of D. pseudo-obscura.

3. The time of pigment deposition in the testes of D. pseudo-obscura, practically coincides with the late phase of eye pigment development. Testis pigment appears a few hours earlier than late phase eye pigment.

4. The amount of pigment in the testes of the various mutants is correlated with the amount of pigment laid down in the eye during the late phase of development and is in no way related to the amount deposited in the eye during the early phase of development.

5. Genes which affect late phase pigment in the eye have the same effect upon testis colour. Those which affect late phase eye pigment quantitatively also affect the testis pigment quantitatively, sepia (se) which has a qualitative effect upon late phase eye pigment has a similar qualitative effect upon testis pigment.

Description of Plate.

Plate I. shows the colours of eyes and testes of various mutants in D. pseudo-obscura. It illustrates that while the eye colour in the purple series, rust and sepia, is affected by the presence of vermilion or orange, the testis colour is unaffected. Testis and eye colour of the eosin-rust gene combination is lighter than that of either eosin or rust. It is due to a summation effect of two genes which affect testis colour late phase eye-pigment development probably at different times (Cochrane 1937 unpublished.)

A series of yellow testis colour dilutions is shown, which are the result of the action of the sepia gene in combination with other genes. These dilutions correspond to parallel variations in the testis and late phase eye-colour of the phenotypes of the single genes when not so combined.

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Appendix.

Spectroscopic examinations of eye-colour extracts have been made by Schultz (1929 and 1935) who found that red and yellow pigments in D. melanogaster have slightly different absorption spectra. This difference suggested that pigment distribution in Drosophila could be analysed by photographic means with the aid of a series of coloured filters. It was hoped in this way to obtain objective data for comparing various eye-colour mutants of D. pseudo-obscura.

For this purpose a photomicrographic camera was constructed which, when set up over the microscope, made it possible to view the image before and after exposure of the film. This facilitated accuracy of focussing and permitted the taking of a number of photographs without removal of the camera. A simple sliding shutter allowed exposure of the film in the open position by means of an aperture. It also carried a prism which, in the closed position, deflected the light rays from the microscope through a side tube at right angles to the long axis of the camera. This side tube was fitted with an ocular similar to that in the microscope and so adjusted that the image was in focus on viewing through the side tube and also in focus on the film when exposed.

Both Agfa Isopanchromatic 17/10 Din. roll-film and Kodak Commercial Panchromatic cut-film were used

for experiment and the former, proving satisfactory for the purpose, was adopted as the standard material.

A thorium pastille gas lamp with a one inch condenser was found to be the most suitable source of light.

Wratten gelatin colour filters were fitted in the sub-stage of the microscope, the series selected after experiment being:- C (blue) B (green) G (orange) A (orange-red) F (red). These had transmission bands shown in the table (taken from "Photomicrography" Kodak Limited 1926)

Filter	Transmission band in Angstrom units
C	From 4,000 to 5,100
B	" 4,600 to 6,000
G	" 5,100 to red end
A	" 5,800 to red end
F	" 6,100 to red end

Photographs were taken using a Reichert 1/12 inch oil immersion objective and a x 12 compensating ocular, with a camera extension of 10 inches.

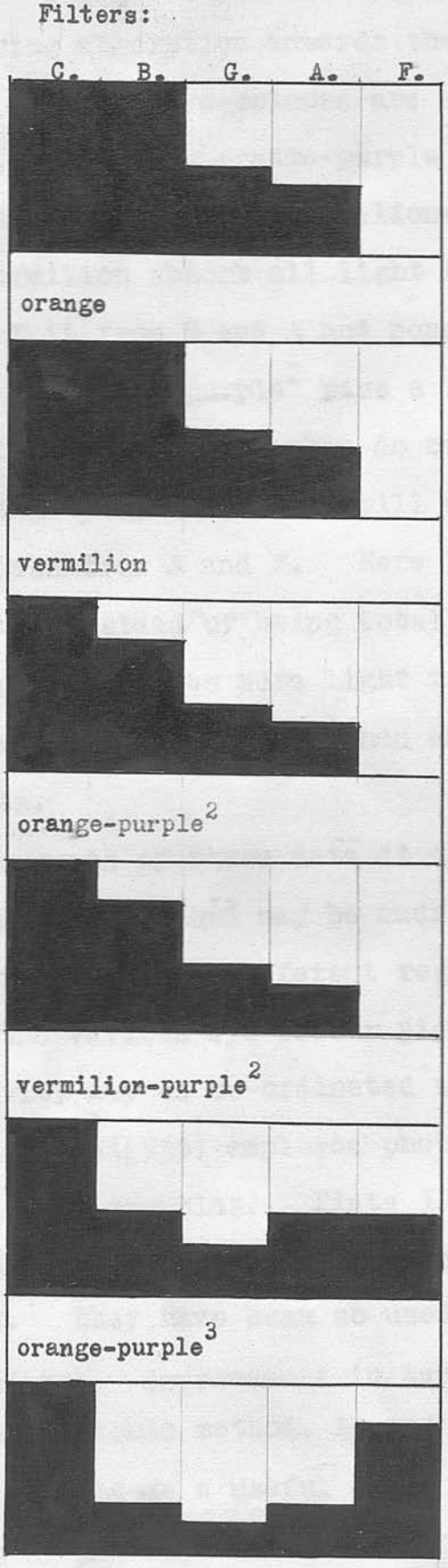
A series of eye-colour mutants was selected showing a range from red to yellow as far as this was possible. Orange and vermilion appeared to contain less red: orange-purple3 still less red and vermilion-sepia yellow pigment. These mutants have one and only one type of granules present in their eyes.

Both sections of eyes and smears from fresh eyes

were examined. Smear preparations were found to be preferable as the granules were there seen in one plane.

The photomicrographs show some differentiation between these pigment colours although difficulty was experienced in obtaining clear images in all the types of granules used. Figure I and Plate I illustrate the amounts of light absorbed by the granules when photographed by light transmitted through the different filters.

Fig. 1. Diagram to show relative absorption of light from a series of filters by various eye-colour pigments.



It will be seen that a series is obtained showing a decreasing absorption towards the blue end of the spectrum when the eye-colours are arranged in the order orange, vermilion, orange-purple^2, vermilion-purple^2, orange-purple^3, and vermilion-sepia. Thus orange and vermilion absorb all light from filters C and B, part of it from G and A and none from F. Orange-purple^2 and vermilion-purple^2 pass a little light from B and more from G and A than do orange or vermilion. Orange-purple^3 passes still more light from B and C but less from A and F. Here light is partially absorbed instead of being totally transmitted. Vermilion-sepia transmits more light from B and G filters but absorbs more from F than any of the foregoing pigments.

On the grounds of these data it is suggested that a photographic record may be made of the relative absorption of light from different regions of the spectrum by the various eye-colour pigments and that this selectivity may be co-ordinated with the observed colours. Schultz (1935) employed photographs for measuring pigment granules. Plate II. shows that they are useful for making relative comparisons of granule size. They have been so used in the course of these studies. Improvement in technique may allow the photographic method, in spite of its limitations, to become a useful means of analysing the distribution of pigments in the eye-colour mutants of Drosophila.

Plate I. Photographs showing absorption of light from a series of filters by various eye-colour pigments.

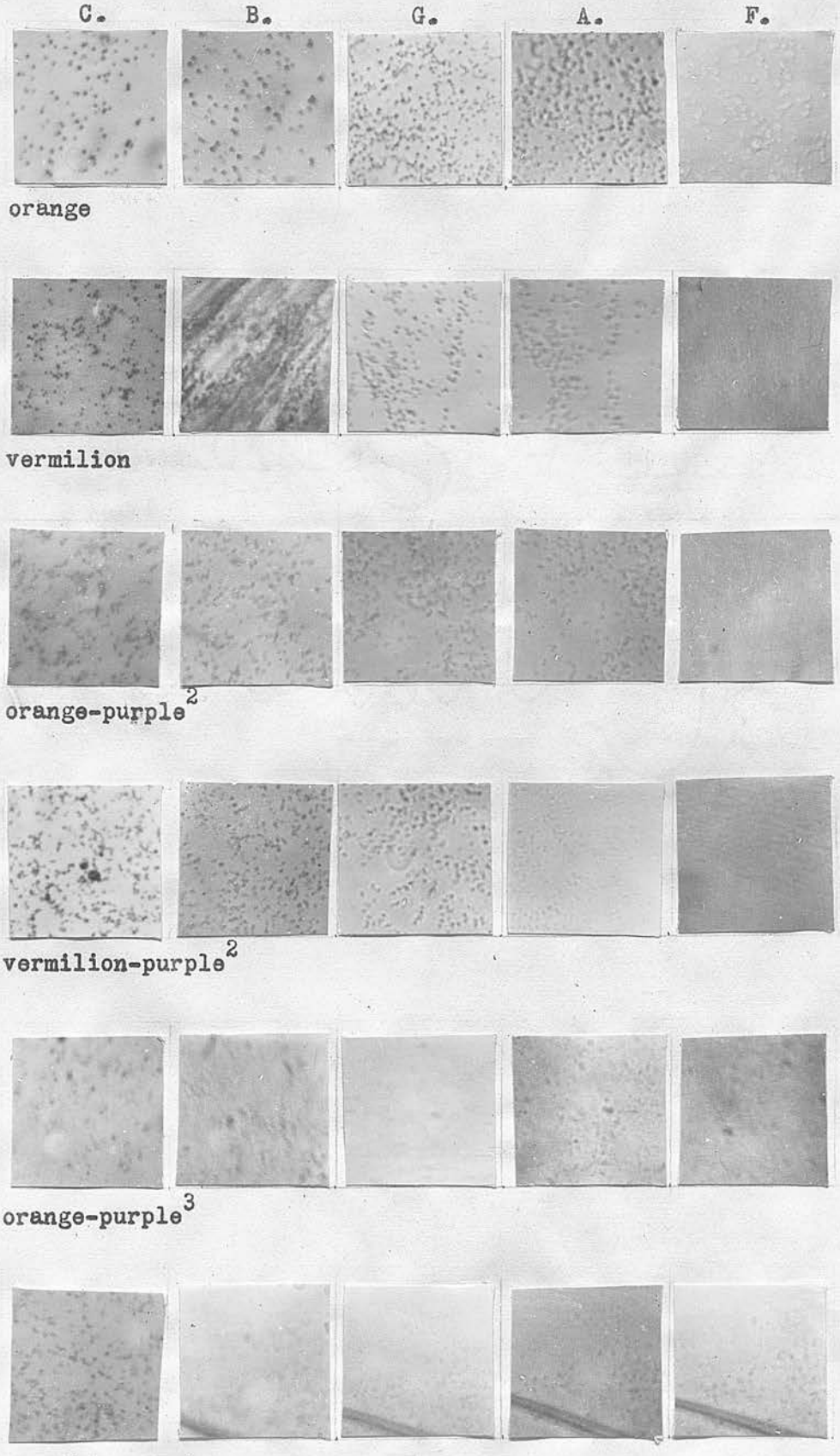
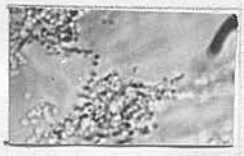


Plate II. Series of photographs showing differences in granule size.

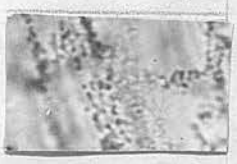
vermilion
adult
2 weeks

vermilion-purple[2]
adult
2 weeks

sepia
adult
2 weeks



orange
adult
young



orange-purple[2]
adult
young

orange-purple[3]
adult
(smear)

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Note with reference to Plates.

The plates will be found in a separate cover as they are of unsuitable size for binding herewith and as they will be required for publication. The expense of making these plates being very great, only one copy is submitted.